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Cryopreserved Thaw and Use Cell Plates: Introducing CryoShield™ and the RoSS.pFTU for Cell Monolayer Cryopreservation

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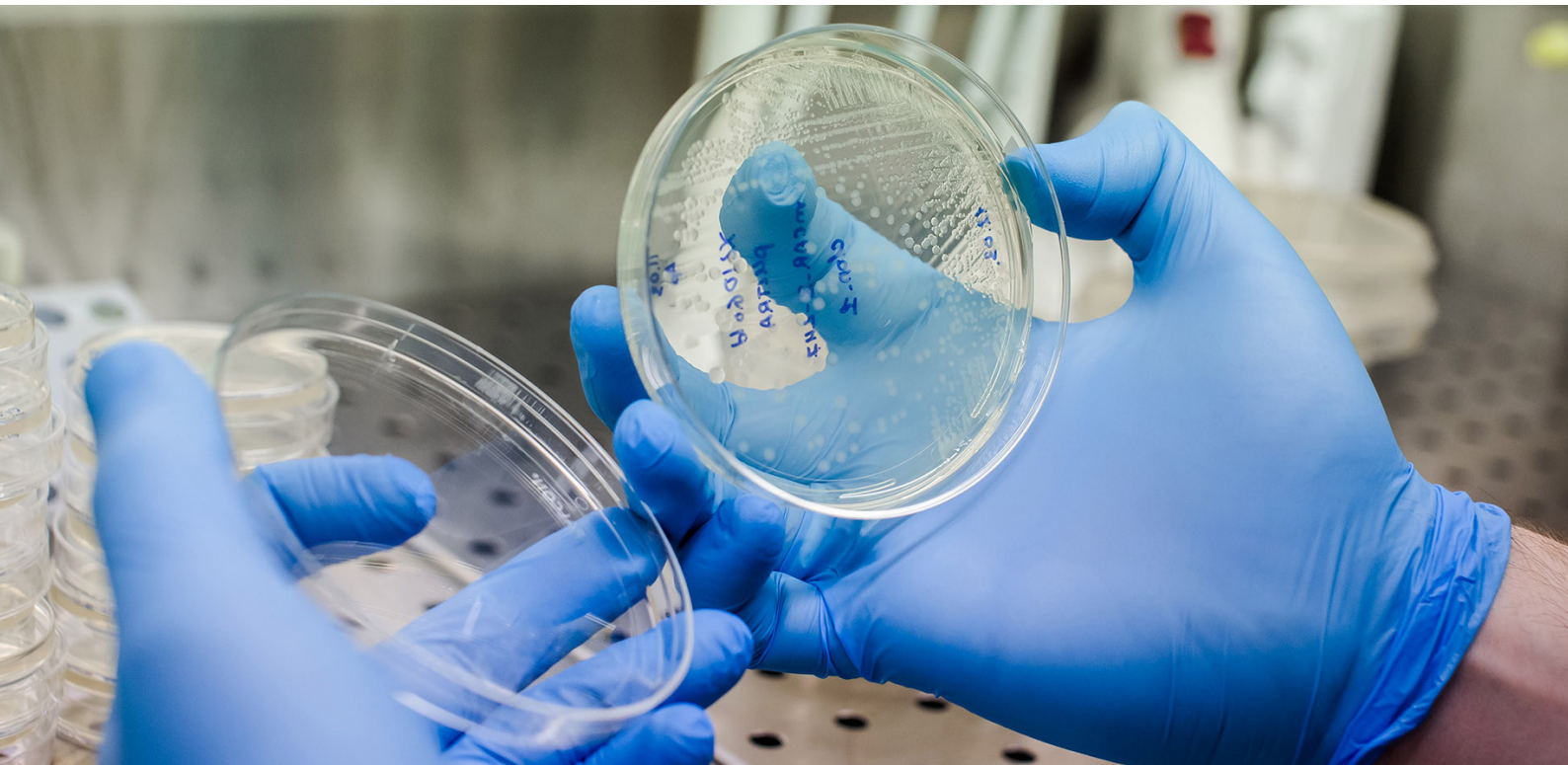
Abstract

Cell monolayers are widely used in the discovery of new drug compounds and the study of cell biology. Current commercially available cryopreservation technologies do not allow cells to be stored frozen whilst attached to tissue culture plastic. Hence, cells must be thawed from a working cell bank, cultured for several days and then transferred into microtiter cell culture plates. This process consumes significant time handling cells, rather than conducting biomedical research or other value-adding activities.

If cells could be successfully cryopreserved directly adhered to microtiter cell culture plates, then the time and in-house technical expertise required to carry out cell-based experiments could be greatly reduced, a financially attractive options for laboratories looking to accelerate their research and expand their scope. For biological research labs of any size, this approach would lower operational risks and overheads caused by cell culture activities. If these cells plates could be stored frozen and then used directly from the freezer, end users would benefit from smaller lab footprints, faster, more flexible research, and consistent data with reduced batch variation compared to using continual cell culture.

Convenient, ready to use from the freezer, cell-based assays could accelerate and simplify the discovery of pharmaceutically active compounds, biocompatibility testing, assay development and discovery of cell signalling and disease pathways. This would speed up product development and time to market.

CryoLogyx has developed a proprietary cryoprotectant technology, CryoShield™ that can successfully cryopreserve cells in adherent formats (Figure 1). It enables routine, reproducible, and robust cryopreservation of biomedically important cell monolayers, within industry-standard tissue culture microtiter plates.[1] The cells are simply thawed with media and placed in an incubator and are then ready to use within hours, compared to DMSO-based cryoprotectants, which require several days of culturing, and produce significant amounts of dead and detached cells which affect experimental results. [2] [3]



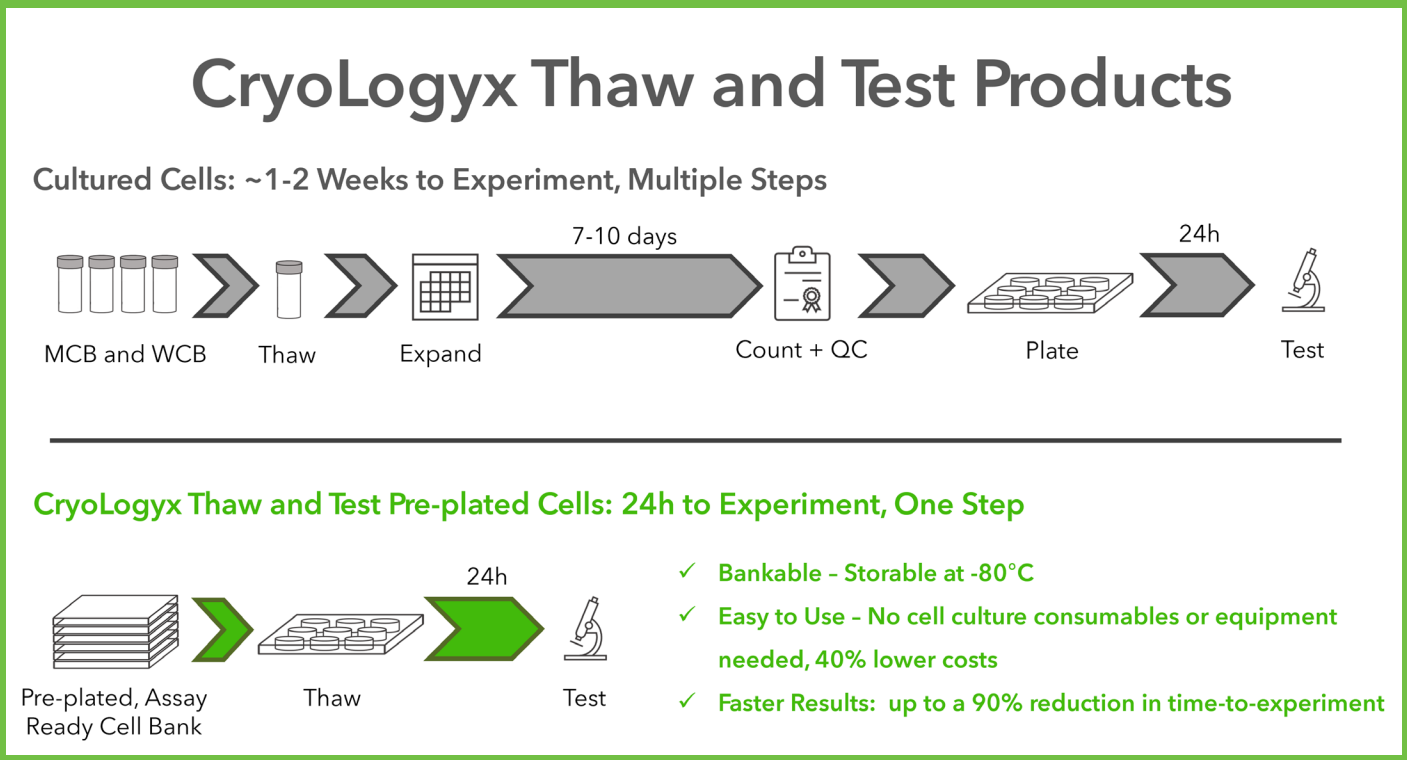


Figure 1. Cryologyx Thaw and Test Products

Figure 1. Conventional approach to cell-based assay workflow, compared to using CryoLogyx Thaw and Test Pre-plated Cells. **Major benefits to users include significant time and cost savings, with the average user seeing a 90% reduction**, from 8-11 days down to 24 hours from initiating lab work to getting cell-based assay data. Consumables costs are significantly reduced, as the product only requires the assay media to be made ready to use, compared to conventional cell culture, which requires large amounts of single use plastic, pipette tips, and cell culture overheads.

Single Use Support's RoSS.pFTU Lab Scale

Single Use Support is a pioneering biopharma operations specialist. Specialising in controlled, scalable freezing process solutions, **Single Use Support has developed the RoSS.pFTU, a plate-based freeze/thaw unit, the perfect solution for clinical studies conducted in labs and for the development of controlled, cGMP-compliant, and scalable freezing processes.**

The volume of cell-based assays being carried out in labs all over the world has dramatically increased in the last decade and looks to grow exponentially in the next few years. In just the UK alone, in the last year over 80,000 HepG2 toxicity challenge assays are carried out in monolayer format in 96 well microtiter plates. This is just from a single cell line, from one country. **The ability to prepare cryopreserved assay ready plates at a scale to meet this demand requires equipment designed for achieving consistent high quality cell culture and freezing.** In this application note we will discuss how CryoShield™ cryoprotectants and the RoSS.pFTU can be uniquely combined to prepare Cryopreserved Thaw and Use Cell Plates.

What is a Successful Cryopreservation?

The effectiveness of cryopreservation solutions is generally only evaluated by measuring post thaw cell viability (the ratio of live cells to total cells post-thaw, this is commonly reported), but this only tells a small part of the story. Firstly, the time at which this viability is measured is vitally important. Measuring directly post thaw will give an artificially high result, as many cells will subsequently die through cryoprotectant-related toxicity, long-term post thaw damage causing apoptosis, or other programmed cell-death pathways. Cell growth, proliferation time, and assay performance should also be measured as there are many freeze/thaw induced issues that can affect cell behaviour, without causing cell death.

Finally, for assay ready applications, the exact number of live cells recovered (typically referred to as the cell recovery) must be consistent across several batches, uniform across the assay format (e.g., a microtiter plate) and contain a minimum of cryoprotectant and dead cells or cellular debris, to provide a truly assay-ready product to virologists, toxicologist, cell biologists and any other researcher that routinely or occasionally works with cells and tissues.

CryoShield™ and RoSS.pFTU Cryopreservation

A549 cells were plated on 24 well tissue culture (TC) microtitre plates and allowed to adhere, then were cryopreserved using conventional DMSO-based cryoprotectants and the freezing process developed by Single Use Support and CryoLogyx. The cell recovery of cell monolayers frozen with conventional cryoprotectants is very low, with significant cell death occurring and many cells detaching from the plate surface. **When the CryoShield™ process and the RoSS.pFTU was used to cryopreserve the cell monolayers, total cell recovery was significantly higher, with greater than 122% recovery (compared to pre-freeze seeding numbers) achieved after 24 hours (Figure 2).** This is due to the cells incurring minimal freeze/thaw induced damage, quickly recovering, and then dividing. LIVE/DEAD™ staining (Figure 3) shows that the cells remain with membranes intact (green cells). [1] Highly reproducible cell recoveries are obtained, minimising any potential well-to-well variation in cell recovery.

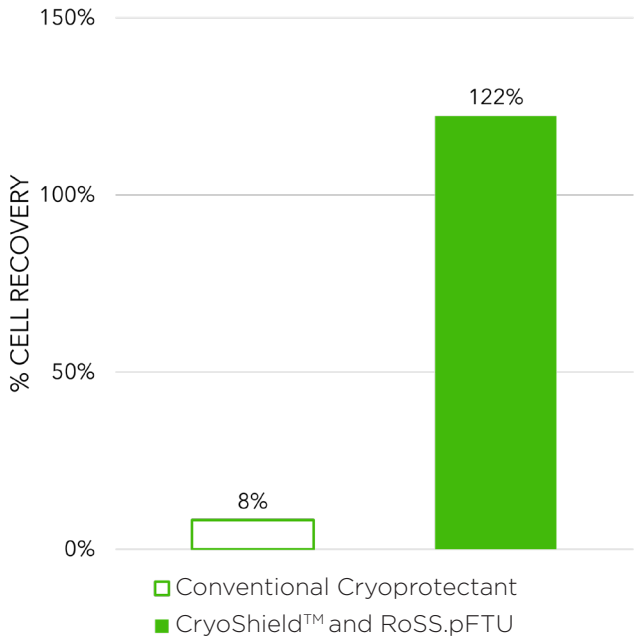


Figure 2. Cell recovery, measured as a percentage of adhered cells in monolayer format recovered with intact membranes from plates post thaw, compared to initial number seeded. Representative data from 3 different wells on a 24 well plate, seeded with 20k cells per well. Conventional cryoprotectant used standard cryopreservation protocols. CryoShield™ and RoSS.pFTU cells were cryopreserved with CryoShield™ cryoprotectant and the RoSS.pFTU controlled rate freezer.

Figure 2. Cell Recovery with and without CryoShield™ and RoSS.pFTU

Figure 3. Cells with intact membranes of A549 and HepG2 cells before and after cryopreservation. LIVE/DEAD assay showing live cells (green) and dead cells (red) post thaw. Recovery shown as percentages (left) and fluorescence microscopy images of overlaid live and dead cells (right).

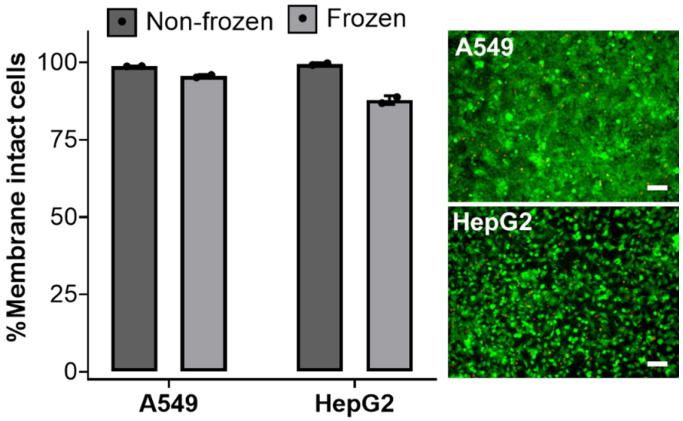


Figure 3. Membrane intact cells

Caspase activation, an indicator of programmed cell death, was minimised confirming that cell health remains excellent post thaw (*Figure 4*). Cell cycle, morphology and growth rates are only minimally affected, making frozen plated cells ideal for screening drug candidates. **Cells cryopreserved with CryoShield™ frozen in the RoSS.pFTU displayed comparable performance to freshly prepared monolayers.** The metabolic function of A549 cells is undisturbed, tested by resazurin reduction to resorufin (*Figure 5*).

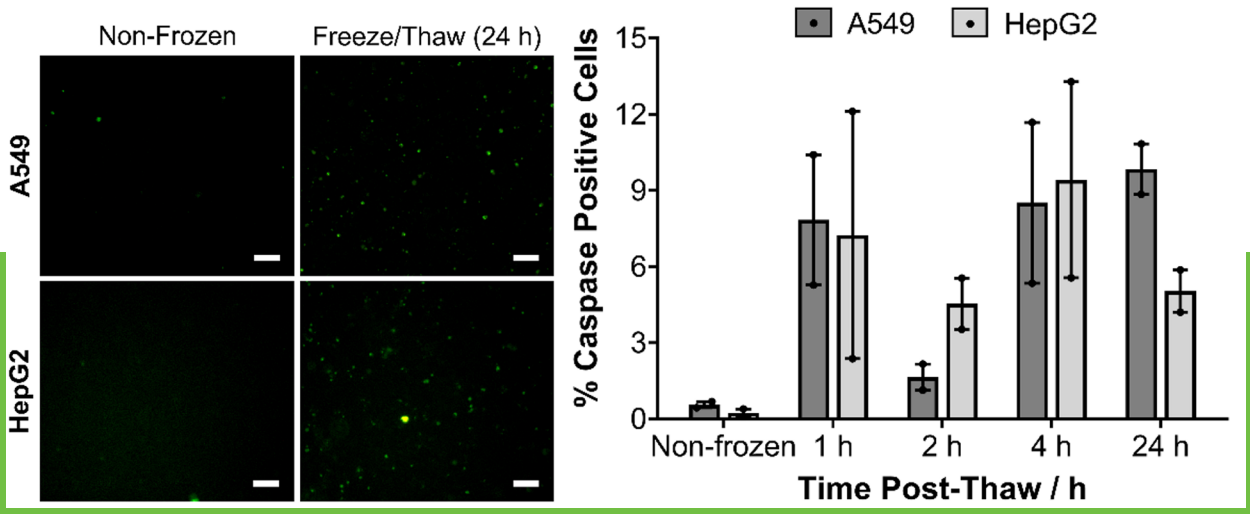


Figure 4. Caspase positive A549 and HepG2 cells

Figure 4. Caspase positive A549 and HepG2 cells; indicative of cells about to undergo apoptosis. Fluorescence microscopy images of caspase positive cells in green (right) and percentage of positive cells per well (left).

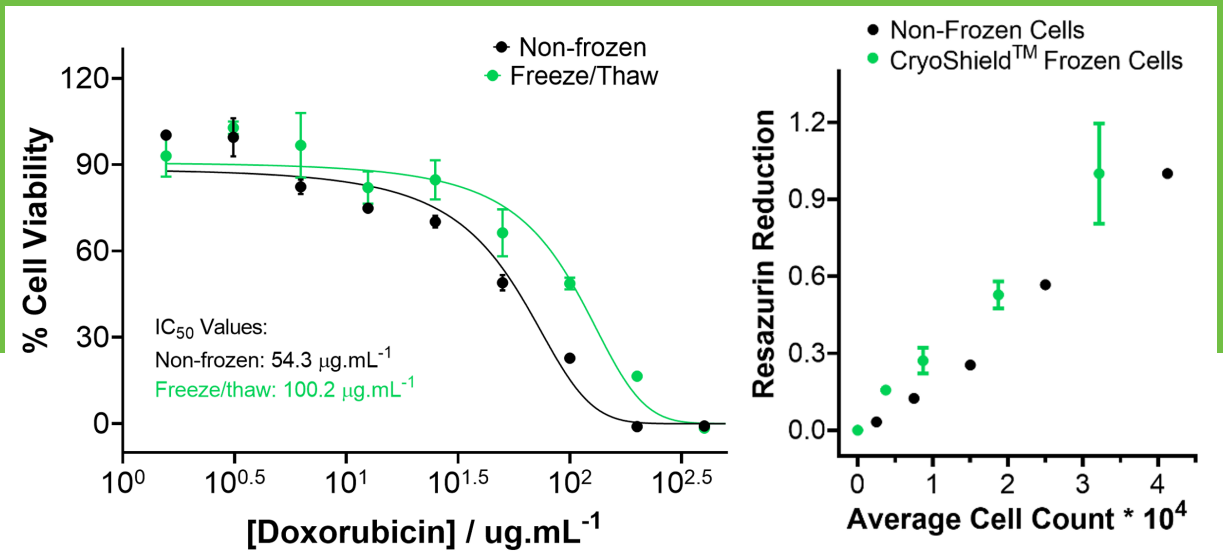


Figure 5. Response from non-frozen and cryopreserved cells

Figure 5. Model assay data showing response from non-frozen and cryopreserved cells. A model toxicity challenge assay using doxorubicin, measuring cell viability at specific concentrations of the drug compound (left). Resazurin reduction at specific cell counts, showing linear response.

Applications & Advantages for Users

The immediate advantage for users is on demand access to cell based assays, without preparatory culture, plating, or handling. Reducing the skill and time required to carry out in vitro research has a huge knock-on effect on research output and infrastructure needed, meaning that more work can be carried out economically in house rather than being outsourced.

For the directors and managers of larger laboratories, the ability to produce and store experiments provides enormous benefits in normalising and simplifying workflows, allowing for surge capacity, and increasing the range of experiments they can carry out. In large institutions cell culture is a time-dependent factor that needs to be worked around, and is incredibly costly to maintain, especially is delays in the lab's workflow mean that prepared batches must be discarded, and the cell culture process restarted.

And finally, for cell line providers and cell banks, frozen, pre-plated assay ready cells open up a whole new market and product line. Many scientists and engineers working at the periphery of biology want to carry out in vitro work but cannot access the skills and equipment needed for a cell culture lab. The requirements for using pre-plated assay ready cells made from cell lines with BSL 1 classification are low, and the experience required even lower, as assay development and optimisation would be carried out by the end user.

Conclusion

‘Thaw-and-Use Assay Cells’ made using CryoShield™ and Single Use Support‘s technologies are a highly effective solution for a range of cell-based assays, from transfection studies to running toxicity screening experiments for drug candidates. No specialised equipment is required by the end user for the thawing procedure, they simply remove the protective film, add cell media, and incubate overnight. Overall, we have shown that cells can be cryopreserved as ‘assay-ready’ monolayers, in a scalable and versatile format. This will enable the development of new screening technologies and enhance automation as well as reduce the experimental burden on researchers, and ultimately enable the highest quality models to be made available to anyone.

Bibliography

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